Drug Development and Industrial Pharmacy, 34:627–631, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040701833948

informa
healthcare

In Situ Intestinal Absorption of Cyclosporine A Solid Dispersion in Rats

Chen Liu, Saijie Zhu, Ying Zhou, Yupu Wei, and Yuanying Pei

Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai, People's Republic of China

Effects of concentration of Polyoxyethylene (40) stearate, Na^+ and P-gp inhibitor on cyclosporin A (CyA-SD) absorption were investigated by in situ circulation method. The results showed that the absorption of CyA increased linearly with its concentration, indicating a passive diffusion process was dominated. CyA absorption decreased with the carrier concentration. The concentration of Na^+ didn't influence the drug absorbed ($P > 0.05$). The P-gp inhibitor enhanced the CyA absorption significantly ($P < 0.05$). The passive diffusion process during the intestinal absorption indicated that the solubility enhancement of CyA is one of the mechanisms for the absorption of this water insoluble drug.

Keywords cyclosporine A; solid dispersion; polyoxyethylene (40) stearate; in situ circulation; absorption behavior

INTRODUCTION

Cyclosporine A (CyA) is a lipophilic drug widely used in the therapy of immunorepulsion after organ transplantation. The solubility of CyA in water is 7.3 μg/mL at 37°C (Ismailos, Reppas, Dressman, & Macheras, 1991). It has a high octanol-water partition coefficient (log P = 2.92, Taylor et al., 1993). According to the biopharmaceutical classification system (Amidon, Lennernäs, Shah, & Crison, 1995), CyA is an example of a class II compound (Chiu et al., 2003), meaning that its oral bioavailability is determined by dissolution rate in the gastrointestinal fluid. Solubility enhancement is one of the effective ways to improve the oral absorption of this kind of drug. Our preliminary experiments have shown that the solubility of CyA can be improved dramatically in aqueous solutions of polyoxyethylene (40) stearate (PS) (Liu, Zhu et al., 2006). The pharmacokinetic studies also demonstrated that the solid dispersion of CyA prepared from PS was bioequivalent to Sandimmum Neoral®, a microemulsion formulation of CyA, in rats (Liu, Wu et al., 2006).

Address correspondence to Yuanying Pei, Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai 200032, P. R. China. E-mail: yypei@shmu.edu.cn

In the present study, an attempt is made to elucidate the absorption mechanism of CyA from solid dispersion by the in situ circulation method. For this purpose, we have investigated effect of CyA, PS concentration, Na^+ concentration, and P-gp inhibitor on the absorption of CyA-SD.

Intestinal absorption characteristics of drugs are very important for oral drug delivery system. There are several methods extensively used to investigate the intestinal absorption behavior of drug compounds in the form of in situ circulation method, intestinal loops, isolated mucosa, everted sacs, and Caco-2 cell lines (Barthe, Woodley, & Houin, 1999; Barthe, Woodley, Kenworthy, & Houin, 1998; Berggren et al., 2004; Boisset et al., 2000; Rodríguez-Ibáñez et al., 2003; Yu, Lipka, Crison, & Amidon, 1996). The information, including the barrier function of epithelium, on drug absorption, kinetic data, effective absorption segment, absorption mechanism, and the factors influencing drug absorption can be obtained by using these methods properly. Among them, the in situ circulation method is carried out in anesthetized animals with the aid of the in situ rat gut perfusion technique, which normally provides very realistic absorption rate. Moreover, the in situ circulation method is considered to be the choice for absorption mechanism studies since the intestinal manipulation is minimal, the aqueous diffusion layer remains unchanged, and normal blood supply is maintained, leading to perfect sink conditions throughout the test. Furthermore, the correlation between the in situ absorption and the absorption in human beings has been verified (Fagerholm, Johansson, & Lennernäs, 1996).

MATERIALS AND METHODS

Materials

CyA (Lot#: 0404901) was a generous gift of Taishan Pharmaceutical Company, Ltd. (Guangdong, China). Acetonitrile UV was obtained from Burdick & Jackson (Muskegon, MI, USA). Methanol of HPLC grade was purchased from Hanbang Chemical Co. (Jiangyin, Jiangsu, China). Water was ultra filtered through a Millipore filtration system (Milli-Q®).

Polyoxyethylene (40) Stearate of Chinese Pharmacopoeia grade was purchased from Nanjing WELL Chemical Corporation, Ltd. (Nanjing, China). All other chemicals and solvents used were of AR grade.

Preparation of Solid Dispersion

The solid dispersion was prepared by solvent-melt method as described in our previous study (Liu, Zhu et al., 2006). Briefly, CyA in anhydrous ethanol was added to the fused PS. The mixture was then quenched and pulverized after evaporation of ethanol. The powder was stored in glass desiccator at room temperature for further experiment.

Determination of CyA

Concentration of CyA was determined by high-pressure liquid chromatography (HPLC). The HPLC system consisted of a pump (Model LC-2010C, Shimadzu, Japan), a 250 mm long C-18 column (5 μm, ODS-2 Hypersil, ThermoHypersil-Keystone, Bellefonte, PA, USA). The mobile phase was composed of acetonitrile, methanol, and water at the ratio of 44:40:16. Flow rate was 1.5 ml/min. The oven temperature was 55°C. CyA in samples was detected at wavelength of 214 nm. The injection volume was 20 μl. The relative retention time was about 6.5 min. A linear relationship between the CyA concentration in the range of 2.5 to 120 μg/ml and the peak area was found. The calibration was $C = 3.5 \times 10^{-5}A + 0.6215$ ($r^2 = 0.9997$).

Physical Adsorption of CyA

The 10-cm long segment of each intestine region was excised and everted. The segment was tied in both ends and immersed in CyA solutions of known concentrations (193.45 μg/ml) for 2 h at 37°C. The intestine segment was then removed and the drug concentration was detected (C_t) and compared with the original concentration in the solution (C_0). The percentage of drug remained in the solution was calculated from $(C_t / C_0) \times 100\%$.

In Situ Uptake Experiment

Sprague Dawley (SD) rats ♂, weighing from 250g to 300g, were supplied by Shanghai SLAC Laboratory Animal Co., Ltd. (Certificate No.: SCXK (Shanghai) 2003-0003). The rats were fasted for 12 h prior to the experiment but were allowed free access to water. The in situ circulation method was used in these experiments. SD rats were anesthetized with 20% urethane solution (6 mg/Kg). A midline abdominal incision was made and the small intestine was exposed. The bile duct was ligated in order to avoid bile secretion into the perfusate. For the regional absorption of CyA, four intestinal sections were isolated and cannulated (all were 10 cm long): the duodenum, the jejunum, ileum, and colon. Each segment was rinsed with normal saline at 37°C for 20 min until the washing appeared clear. After that, CyA-SD solution of 50 ml with normal saline as solvent was connected to the each segment and recirculated through each part of the four intestine sections for 2 h separately. The circulation rate was 1 ml/min controlled by a peristaltic pump. Finally, PS solution at the concentration of 0.1% was used to perfuse the intestinal segment for 30 min to avoid the adsorption of CyA to the tubes. The perfusate was diluted to 100 ml and the drug remaining in the circulation fluid was detected by HPLC. After perfusion, the corresponding intestine was excised and the area of the segment was measured. For the other experiments, a 10 cm long ileum was isolated and cannulated to examine the absorption of CyA using the method described above. The accumulated drug absorbed in each intestinal section was calculated using the following equation (Ren, Zhang, & Wang, 1997):

$$\text{Drug absorbed per area} = (C_1 \times 50 - C_2 \times 100) / A \qquad (1)$$

Where C_1 and C_2 is the drug concentration before and after circulation and A is the area of the intestine section (cm^2).

Approval for this project was granted by the Institutional Ethical Committee for Animal Experimentation (No.2006-03).

Stability of CyA in the Intestine Circulation Fluid

The whole intestine was isolated and cannulated for circulation. The normal saline containing 4 mg/ml of PS (200 ml) was recirculated through the whole intestine for 2 h at 37°C to get the blank circulation fluid. Solutions containing CyA at the concentration of 50 and 100 μg/ml were prepared by the blank circulation fluid and allowed to stand at 37°C for 2 h. The CyA concentrations before and after the incubation were determined by HPLC as described above, and the stability was evaluated from the data. All experiments were carried out in triplicate.

Site Dependency of Drug Absorption

The perfusion experiment was carried out as described above. Four intestinal sections including duodenum, jejunum, ileum, and colon were used to investigate the site dependency of CyA absorption. CyA solutions at the concentration of 200 μg/ml prepared from solid dispersion were recirculated through each section of the intestine and the drug that remained in the perfusate at 2 h was determined. The amount absorbed in each section of the intestine was compared and the section with the best absorption of CyA was selected.

Effect of CyA Concentration on Drug Absorption

For solid dispersion, solutions containing different concentration of CyA were prepared by dissolving proper amount of solid dispersion (the ratio of CyA and PS was 1:12) in normal

saline (CyA concentration 50.1–202.5 μg/ml). For physical mixture, CyA was dissolved in normal saline containing 4000 μg/ml of PS to make solutions of different drug concentrations (CyA concentration 58.61–245.07 μg/ml). The CyA solutions made from solid dispersion and physical mixture were perfused through the ileum section for 2 h as described above. The perfusate was diluted to 100 ml and the concentration of CyA was determined by HPLC. The accumulated drug absorbed was calculated and compared.

Effect of PS Concentration on Drug Absorption

In order to evaluate the carrier concentration on drug absorption, CyA solutions containing different concentrations of PS were recirculated through ileum for 2 h. CyA solutions (50 μg/ml) were prepared by dissolving proper amount of solid dispersion in normal saline, PS was then added to the solution to make a final concentration between 400 to 7000 μg/ml. After circulation, the drug absorbed was calculated and compared.

Effect of Na^+ on CyA Absorption from Solid Dispersion

The effect of Na^+ concentration on the absorption was conducted by lowering the concentration of Na^+ from 154 mM in the normal saline to 20 mM. K^+ was added to compensate the osmolarity of the solution. CyA solutions (200 μg/ml) were prepared by using the saline containing 20 mM Na^+ as solvent and circulated through ileum for 2 h using the method mentioned above. The concentration of CyA in the perfusate was determined by HPLC and the amount of drug absorbed was compared with that of the sample prepared by the normal saline.

Effect of P-gp Inhibitor on CyA Absorption

Quinidine is known to inhibit P-gp (Ford & Hait, 1990). Quinidine (final concentration 50 μM) was added to the CyA (200 μg/ml) containing circulation fluid and then the fluid was circulated for 2 h using the above mentioned method. CyA concentration in the perfusate after circulation was determined and the amount absorbed was calculated. The difference of the drug absorbed with the presence of P-gp inhibitor was compared with that without Quinidine.

RESULTS AND DISCUSSION

Physical Adsorption of CyA

The physical absorption and stability tests were performed in order to be sure that the drug disappearance from the luminal content was only due to genuine absorption. The adsorption of CyA to the filter membrane was found during our previous experiments and the adsorption of CyA onto the inner polystyrene surface of Transwell® was reported elsewhere (Lee, Chung, & Shim, 2001). Previous tests verified that 0.1% (W/V) PS solution was effective to prevent the adsorption of CyA onto the silicone tubes. To avoid the adsorption, glassware was used through the tests. After incubation with the excised intestinal section at 37°C, the percentage of drug that remained for duodenum, jejunum, ileum, and colon was 98.67 ± 0.07%, 98.01 ± 0.38%, 99.44 ± 0.23%, and 100.77 ± 0.12%, respectively. These results indicated that the physical absorption of CyA to the lumen is negligible.

Stability of CyA in the Intestine Circulation Fluid

Recovery of CyA from the blank circulation fluid was conducted by adding known concentration of CyA in the medium and kept at 37°C for 2 h. The recovery of CyA was 98.42 ± 0.84%, indicating that the circulation fluid did not disturb the analysis of CyA and that CyA was stable in the circulation fluid during the experimental period.

Site Dependency of Drug Absorption

Figure 1 displayed the site dependency of CyA absorption. It is shown in Figure 1 that there is no significant difference in the accumulated drug absorption among duodenum, jejunum, and ileum ($P > 0.05$). The drug absorption from these three sections was much higher than that from the colon ($P < 0.01$). The results illustrated that CyA was mainly absorbed from the upper part of intestine rather than the colon. Therefore, ileum was selected for the evaluation of the effect of drug concentration, PS concentration, Na^+, and P-gp inhibitor on the absorption of CyA.

Effect of CyA Concentration on Drug Absorption

In both cases, the accumulated drug absorption within 2 h increased with CyA concentrations. A linear relationship was observed between the accumulated drug absorbed and CyA concentration (Figure 2). More absorption of CyA from solid dispersion than physical mixture was observed at the same

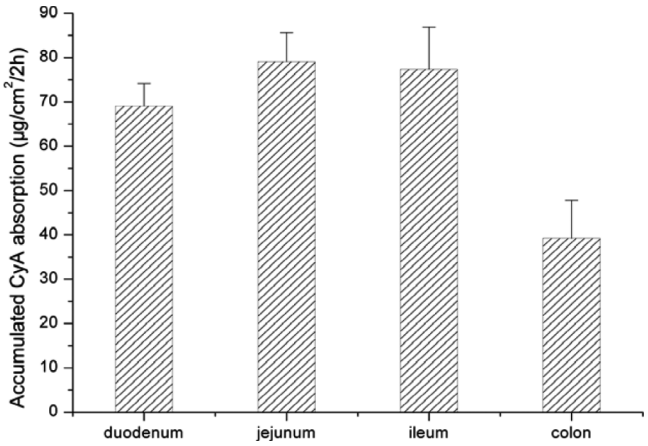

FIGURE 1. Site dependency of CyA absorption.

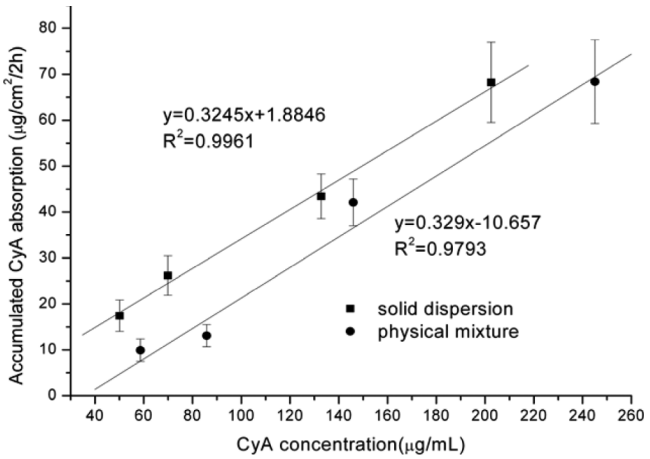

FIGURE 2. Effect of CyA concentration on drug absorption from solid dispersion (■) and physical mixture (•).

drug concentration. The results illustrated that the transport of CyA through ileum at the concentration ranged from 50 to 200 μg/ml for solid dispersion and from 35 to 245 μg/ml for physical mixture was a passive diffusion process.

CyA is a well-known substrate of P-glycoprotein (P-gp, Wacher, Wu, & Benet, 1995). P-gp is located on the apical membrane of the intestinal cells and acts as a counter-transport pump which actively transports CyA back to the intestinal lumen as they are absorbed across the intestinal mucosa (Hebert, 1997; Watkins, 1997). During the intestinal absorption, CyA is partly transported out of the cell by P-gp. Therefore the apparent absorption of CyA is the comprehensive effect of passive diffusion process and the active efflux by P-gp. The human Caco-2 cell was previously used as an in vitro intestinal barrier model, and the uptake of CyA into these cells could occur by passive diffusion (Fricker et al., 1996). In the presence of P-gp, the uptake of CyA is mediated by P-gp, and P-gp can be saturated. When P-gp-mediated uptake is saturated, passive diffusion becomes the rate-limiting step in CyA absorption (Fricker et al., 1996). This model helps explain the observation in this experiment that an increased CyA concentration can lead to increases in the extent of CyA absorption. Solubility of CyA in water was significantly increased by the formation of solid dispersion. The high concentration of CyA in the intestinal lumen may saturate the intestinal P-gp, thus the dominated passive diffusion process was observed in this experiment as shown in Figure 2. At the same time, the surfactant PS used in this system increases the drug solubility by the formation of micelles (Liu, Zhu et al., 2006). The effect of this micellar solubilization is to accelerate diffusion through the unstirred layer coating the intestinal mucosa and to carry the drug efficiently into enterocytes. The results of our experiment indicated that solubility enhancement of CyA by solid dispersion technique and the use of PS as a carrier are the reasons to improve the absorption of this water insoluble drug.

Effect of Carrier Concentration on Drug Absorption

The relationship between the accumulated CyA absorption and the concentration of PS is shown in Figure 3. It was found that the accumulated drug absorption decreased with the increase of the PS concentration within the range of 400 to 7000 μg/ml.

The effect of PS concentration on drug absorption indicated that the accumulated CyA absorption was inversely related to the PS concentration. Similar finding was reported that the transport of poor water soluble drug UC-781 decreased when the concentration of surfactant VitE-TPGS was higher than its critical micelle concentration (CMC) (Deferme et al., 2002). It is reported (Lo, 2003) that PS can inhibit the efflux of intestinal P-gp at the concentration below CMC. Since the PS concentrations used in this experiment are far above its CMC (168.4 μg/ml), the inhibitive effect of PS may be negligible. The total concentration of CyA is composed of free drug and drug in the micelle. The micelles are in equilibrium with free solutes, and the actual absorption is due to partitioning of free solute into the membrane. When the free drug was absorbed, the drug in the micellar phase will partition into the solution to keep the equilibrium. An increase in PS concentration in water will lead to an increase in the amount of micelles or the enlargement of the micelle volume, which will then lower the partition rate of CyA and decrease the fraction of drug free in solution. Thus, the thermodynamic activity of drug, that is, the driving force for drug permeation decreased after they are incorporated into micelles with high concentration of PS. According to Collett et al. (1973), salicylic acid in the micellar phase was practically unavailable for absorption in a reasonable length of time. It may also happen to CyA when PS at a concentration of 7000 μg/ml was used, in which very low absorption of CyA was observed. The model also is confirmed by the finding that more CyA was absorbed from solid dispersion than from physical mixture where much more PS was needed to dissolve same amount of CyA (Figure 2).

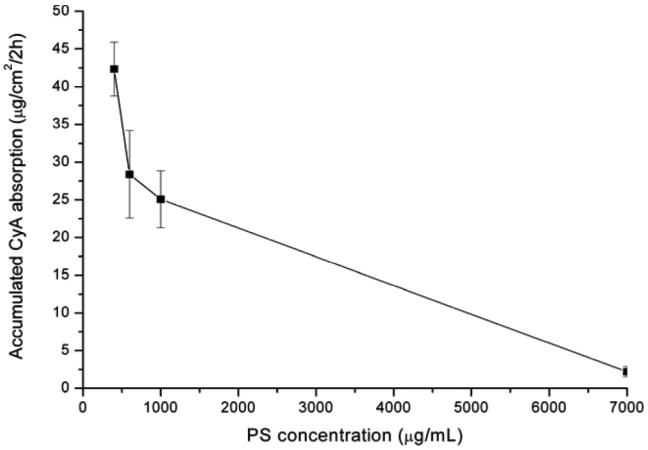

FIGURE 3. Effect of PS concentration on CyA absorption.

Effect of Na^+ on CyA Absorption from Solid Dispersion

The accumulated drug absorption changed from 68.47 ± 9.51 μg/cm^2/2 h to 72.53 ± 8.03 μg/cm^2/2 h when the concentration of Na^+ lowered from 154 mM to 20 mM. There is no statistical significance between the two groups ($P > 0.05$), indicating that the Na^+ concentration has no influence on CyA absorption.

Effect of P-gp Inhibitor on CyA Absorption

The accumulated amount of CyA absorption from solid dispersion in the presence and absence of P-gp inhibitor, Quinidine, was 110.7 ± 15.37 μg/cm^2/2 h and 68.47 ± 9.51 μg/cm^2/2 h, respectively. The absorption of CyA was increased dramatically with the presence of Quinidine in the circulation fluid ($P < 0.05$), indicating that intestinal P-gp was competitively inhibited by Quinidine, thus increased the CyA absorption.

CONCLUSION

In conclusion, CyA had an effective absorption in duodenum, jejunum, and ileum when prepared into solid dispersion with PS. The concentration of Na^+ didn't affect the accumulated CyA absorption significantly ($P > 0.05$). With the presence of P-gp inhibitor, Quinidine, in the circulation fluid, the accumulated absorption of CyA increased significantly ($P < 0.05$). A passive diffusion process was dominated during the intestinal absorption, indicating that the solubility enhancement of CyA is one of the mechanisms for the improved absorption of this water insoluble drug.

REFERENCES

Amidon, G. L., Lennernäs, H., Shah, V. P., & Crison, J. R. (1995). A theoretical basis for biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res., 12*, 413–420.

Barthe, L., Woodley, J., & Houin, G. (1999). Gastrointestinal absorption of drugs: Methods and studies. *Fundam. Clin. Pharmacol., 13*, 154–168.

Barthe, L., Woodley, J. F., Kenworthy, S., & Houin, G. (1998). An improved everted gut sac as a simple and accurate technique to measure paracellular transport across the small intestine. *Eur. J. Drug. Metab. Pharmacokinet., 23*, 313–323.

Berggren, S., Hoogstraate, J., Fagerholm, U., & Lennernäs, H. (2004). Characterization of jejunal absorption and apical efflux of ropivacaine, lidocaine and bupivacaine in the rat using in situ and in vitro absorption models. *Eur. J. Pharm. Sci., 21*, 553–560.

Boisset, M., Botham, R. P., Haegele, K. D., Lenfant, B., & Pachot, J. I. (2000). Absorption of angiotensin II antagonists in Ussing chambers, Caco-2, perfused jejunum loop and in vivo: Importance of drug ionization in the in vitro prediction of in vivo absorption. *Eur. J. Pharm. Sci., 10*, 215–224.

Chiu, Y. Y., Higaki, K., Neudeck, B. L., Barnett, J. L., Welage, L. S., & Amidon, G. L. (2003). Human jejunal permeability of cyclosporin A: Influence of surfactants on P-glycoprotein efflux in Caco-2 cells. *Pharm. Res., 20*, 749–756.

Collett, J. H., Withington, R., & Price, P. H. (1973). An analogue computer study of the dialysis of salicylic acid from micellar solutions of polysorbate 20 and 80. *J. Pharm. Pharmacol., 25*, 974–981.

Deferme, S.,Van Gelder, J., Ingels, F., Van den Mooter, G., De Buck, S., Balzarini, J., Naesens, L., De Clercq, E., Kinget, R., & Augustijns, P. (2002). Intestinal absorption characteristics of the low solubility thiocarboxanilide UC-781. *Int. J. Pharm., 234*, 113–119.

Fagerholm, U., Johansson, M., & Lennernäs, H. (1996). Comparison between permeability coefficients in rat and human jejunum. *Pharm. Res., 9*, 1336–1342.

Ford, J. M., & Hait, W. N. (1990). Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol. Rev., 42*, 155–199.

Fricker, G., Drewe, J., Huwyler, J., Gutmann, H., & Beglinger, C. L. (1996). Relevance of P-glycoprotein for the enteral absorption of cyclosporin A: In vitro-in vivo correlation. *Br. J. Pharmacol., 118*, 1841–1847.

Hebert, M. F. (1997). Contribution of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. *Adv. Drug. Deliv. Rev., 27*, 201–214.

Ismailos, G., Reppas, C., Dressman, J. B., & Macheras, P. (1991). Unusual solubility behavior of cyclosporin A in aqueous media. *J. Pharm. Pharmacol., 43*, 287–289.

Lee, Y. J., Chung, S. J., & Shim, C. K. (2001). The prevention of cyclosporine A adsorption to Transwell® surfaces by human plasma. *Int. J. Pharm., 224*, 201–204.

Liu, C., Wu, J. H., Shi, B., Zhang, Y. X., Gao, T. K., & Pei, Y. Y. (2006). Enhancing the bioavailability of Cyclosporine A using solid dispersion containing polyoxyethylene (40) stearate. *Drug. Dev. Ind. Pharm., 32*, 115–123.

Liu, C., Zhu, S. J., Zhou, Y., Wei, Y. P., & Pei, Y. Y. (2006). Enhancement of dissolution of cyclosporine A using solid dispersions with polyoxyethylene (40) stearate. *Pharmazie., 8*, 681–684.

Lo, Y. L. (2003). Relationships between the hydrophilic-lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines. *J. Control. Rel., 90*, 37–48.

Ren, X. Z., Zhang, J. S., & Wang, H. B. (1997). Studies of absorption mechanism and the best absorption segment of Erythromycin in situ. *J. Chin. Pharm. Univ., 28*, 17–22.

Rodríguez-Ibáñez, M., Nalda-Molina, R., Montalar-Montero, M., Bermejo, M. V., Merino, V., & Garrigues, T. M. (2003). Transintestinal secretion of ciprofloxacin, grepafloxacin and sparfloxacin: In vitro and in situ inhibition studies. *Eur. J. Pharm. Biopharm. Sci., 55*, 241–246.

Taylor, N. E., Mark, A. E., Vallat, P., Brunne, R. M., Testa, B., & Van Gunteren, W. F. (1993). Solvent dependent conformation and hydrogen bounding capacity of cyclosporin A: Evidence from partition coefficient and molecular dynamic simulations. *J. Med. Chem., 36*, 3753–3764.

Wacher, V. J., Wu, C. Y., & Benet, L. Z. (1995). Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy. *Mol. Carcinog., 13*, 129–134.

Watkins, P. B. (1997). The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Adv. Drug. Deliv. Rev., 27*, 161–170.

Yu, L. X., Lipka, E., Crison, J. R., & Amidon, G. L. (1996). Transport approaches to the biopharmaceutical design of oral drug delivery system: Prediction of intestinal absorption. *Adv. Drug. Del. Rev., 19*, 359–376.